Abstracts of Submitted Presentations

S25 CpG ODN is safe and highly effective in humans as adjuvant to HBV vaccine: Preliminary results of Phase I trial with CpG ODN 7909

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Immunostimulatory CpG motif are unmethylated cytosine-guanine dinucleotides common in bacterial DNA but rare in mammalian DNA. CpG DNA activates many immune cells. In animal models, CpG-containing oligodeoxynucleotides (CpG ODN) act as potent Th1-like adjuvants with many antigens. We identified a 24-mer containing 3 CpG motifs (CpG 7909) that strongly activates human cells *in vitro*. In this first report of using CpG 7909 in humans, we evaluated the safety, tolerability and immunogenicity of CpG 7909 as adjuvant to a hepatitis B virus (HBV) vaccine.

In a double-blind phase I study being carried out at two Canadian sites, healthy volunteers aged 18-35 years are vaccinated at 0, 1 and 6 mos by IM injection with Engerix-B® (SmithKline Beecham), which contains 20µg yeast-derived hepatitis B surface antigen [HBsAg] adsorbed to alum, with or without added CpG 7909 (500µg). Interim group-wise comparison shows experimental vaccines, like control vaccines, to be well tolerated, both locally and systemically. However, HBsAg-specific antibody responses (anti-HBs) are significantly better in CpG than control subjects. With control vaccine, no subjects (n=8, to date) had seroconverted (SC, anti-HBs>1 mIU/ml) at 2 wk post-prime and there was only 25% SC and 13% seroprotection (SP, >10 mIU/ml) by 4 wk. In contrast, those receiving CpG 7909 with their vaccines (n=12) had 83 and 92% SC and 58 and 75% SP at 2 and 4 wk respectively. Post-boost (6 wk), SP was 57% for control subjects but 100% for subjects receiving CpG 7909. Anti-HBs titers were significantly higher with CpG, for all time points after prime and boost ($p<0.02$). We are also evaluating antibody isotype to identify the Th1/Th2 bias of responses.

These results indicate that CpG 7909 may allow the development of a 2- rather than a 3-dose HBV vaccine, and that it may be a safe and highly effective adjuvant for a wide variety of human vaccines.

S26 Using C3d to Increase the Immunogenicity of HIV-1 Envelope and Influenza Hemagglutinin in Mice.

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DNA immunogens were used to test the C3d component of complement as a molecular adjuvant for HIV-1 envelope (Env) or Influenza hemagglutinin (HA) immunogenicity. In normal immune responses, the attachment of C3d to an antigen (Ag) enhances both the initiation and maturation of Ag-specific antibody (Ab) responses. The HIV-1 Env has been a target for developing an effective vaccine, however, Env has proven to be a weak immunogen; raising antibodies that are slow to undergo affinity maturation. DNA constructs were produced which encoded soluble gp120 or soluble HA fused to C3d (sgp120-3C3d or sHA-3C3d). The working hypothesis was soluble fusion proteins would increase the immunogenicity of either Env or HA in DNA immunized mice. Our results in mice inoculated with plasmids expressing sHA-3C3d had a 15 fold increase in total IgG to HA compared to mice inoculated with plasmids expressing sHA only. The amount of anti-HA IgG raised in mice inoculated with sHA-3C3d was similar to the amount of antibody raised in mice inoculated with the natural, transmembrane form of HA (tmHA). In mice inoculated with sgp120-3C3d constructs, there was at least 32 fold increase in anti-Env IgG antibody levels compared to mice inoculated with sgp120 only expressing plasmids. The avidity of the anti-HA or anti-Env antibody was 20% higher in mice inoculated with 3C3d fusion plasmids than in sHA or sgp120 only inoculated mice. The level of HAI titers were higher in sHA-3C3d inoculated mice compared to mice inoculated with sHA only. However, these HAI titers were less than the levels seen in mice inoculated with tmHA expressing plasmids. In addition, there was a significant correlation between HAI titer and protection in mice to challenge Influenza virus. This work was funded in part by NIH/NIAID Award R21 AI44325-01 (to T.M.R.).

S27 Evaluation of Carbohydrate-PADRE Peptide Conjugate Vaccine Technology

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The PADRE technology (Pan HLA-DR Epitope) is based on a family of synthetic peptides that were designed to bind to the majority of common HLA-DR antigens with moderate to high affinity, and to present immunogenic, large or charged, amino acids to the T-cell receptors on CD4+ helper T-lymphocytes. In its peptide form, PADRE is highly immunogenic and capable of inducing proliferation of naïve helper T-lymphocytes. Thus, it may be well suited for use as an adjuvant-active carrier for carbohydrate vaccine antigens. To test this hypothesis, we constructed experimental glycoconjugate vaccines based on PADRE and bacterial carbohydrates and evaluated their immunogenicity in C57BL/6 mice. Included were the dodecasaccharide derived from *Salmonella typhimurium* lipopolysaccharide O-antigen and capsular polysaccharides from *Streptococcus pneumoniae*. All of the glycoconjugates induced higher titered antibody responses in mice than did carbohydrates used alone. Different adjuvants, in combination with PADRE conjugates also proved useful and allowed for the modulation of the antibody isotype profile. Alum adjuvant supported predominantly IgG1 responses whereas Qs-21 saponin adjvuant augmented IgG2a, IgG2b titers. The antibodies produced by these vaccines bound to intact bacteria, suggesting that biologically relevant specificities were produced.

S28 Novel Synthetic LPS Receptor Agonists as Vaccine Adjuvants

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Objective: The immunologic analysis of simplified synthetic LPS receptor agonists screened for use as potential vaccine adjuvants.

Methods and Results: LPS receptor agonists consisting of a six-fatty acid chain system with a non-sugar head structure were synthesized. Human whole blood stimulated with the compounds produced IL-10 and TNF-α. Structural modifications result in a series of molecules with a range of cytokine stimulatory activity, all significantly attenuated compared to LPS. The compounds activated an NFKB-driven reporter gene in cells expressing TLR4/MD2 surface molecules.

When mixed in aqueous solution with protein or polysaccharide antigens, the agonists boosted serum antibody responses in mice ten-fold or more above unadjuvanted antigen. Antibody was largely of the IgG1 subclass, indicative of a Th2-associated cytokine pattern. Antibody responses against the adjuvant itself occurred at less than 5% frequency. The compounds also supported antibody responses to tetanus toxoid given intranasally. Nasal and vaginal IgA was elicited in this immunization regimen, in addition to circulating IgG. The compounds showed excellent local safety when given s.c. in mice. High doses resulted in circulating IL-10, but doses below 30 µg did not result in measurable serum IL-10 increases, although retaining their adjuvant effects.

Conclusions: Simplified LPS receptor agonists, which can be synthesized to high purity, have the potential to be efficacious, low-cost adjuvants.